



CLAIMS

What is claimed is:

- Sub B3
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1. A method of cloning an animal, comprising the steps of:
 - a. combining a genome from an activated donor cell with an activated, enucleated oocyte to thereby form a nuclear transfer embryo; and
 - b. impregnating an animal with the nuclear transfer embryo in conditions suitable for gestation of the cloned animal.
 2. The method of Claim 1, wherein the activated donor cell is in a stage of a mitotic cell cycle selected from the group consisting of: G₁ phase, S phase, and G₂/M phase.
 3. The method of Claim 2, wherein the activated donor cell is selected from the group consisting of: a somatic cell, a germ cell and a stem cell.
 4. The method of Claim 3, wherein the somatic cell is an adult somatic cell or an embryonic somatic cell.
 - 15 5. The method of Claim 3, wherein the somatic cell is a fibroblast cell or an epithelial cell.
 6. The method of Claim 1, wherein the activated, enucleated oocyte is in a stage of a meiotic cell cycle selected from the group consisting of: metaphase I, anaphase I, anaphase II and telophase II.

7. The method of Claim 1, wherein the oocyte is enucleated chemically, by X-ray irradiation, by laser irradiation or by physical removal.
8. A method of producing a transgenic animal, comprising the steps of:
- combining a genetically engineered genome from an activated donor cell with an activated, enucleated oocyte to thereby form a transgenic nuclear transfer embryo; and
 - impregnating an animal with the transgenic nuclear transfer embryo in conditions suitable for gestation of the transgenic animal.
9. The method of Claim 8, wherein the activated donor cell is in a stage of a mitotic cell cycle selected from the group consisting of: G₁ phase, S phase, and G₂/M phase.
10. The method of Claim 8, wherein the activated, enucleated oocyte is in a stage of a meiotic cell cycle selected from the group consisting of: metaphase I, anaphase I, anaphase II and telophase II.
11. A method of producing a nuclear transfer embryo, comprising combining a genome from an activated donor cell with an activated, enucleated oocyte.
12. The method of Claim 11, wherein the oocyte is activated by exposing the oocyte to increased levels of calcium.
13. The method of Claim 12, further including decreasing phosphorylation in the oocyte.

14. The method of Claim 13, wherein the oocyte is activated by subjecting the oocyte to ethanol, ionophore or electrical stimulation in the presence of calcium.

15. The method of Claim 11, wherein the oocyte is in Metaphase II prior to activation.

5 16. The method of Claim 11, wherein the donor cell is activated by reducing nutrients in the serum of the donor cell, and then exposing the donor cell to serum having an increased amount of nutrients.

10 17. The method of Claim 16, wherein the activated donor cell is in a stage of a mitotic cell cycle selected from the group consisting of: G₁ phase, S phase, and G₂/M phase.

18. The method of Claim 11, wherein the activated, enucleated oocyte is in a stage of a meiotic cell cycle selected from the group consisting of: metaphase I, anaphase I, anaphase II and telophase II.

15 19. The method of Claim 11, wherein combining a genome from an activated donor cell with an activated oocyte further includes fusing the activated donor cell with the activated oocyte.

20. The method of Claim 11, wherein combining a genome from an activated donor cell with an activated oocyte further includes microinjecting the nucleus of the activated donor cell into the activated oocyte.

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21. A method of producing a protein of interest in an animal, comprising the steps of:
- a. combining a genome from an activated donor cell with an activated, enucleated oocyte to thereby form a nuclear transfer embryo, wherein the genome from the activated donor cell encodes the protein of interest;
- b. impregnating an animal with the nuclear transfer embryo in conditions suitable for gestation of a cloned animal; and
- c. purifying the protein of interest from the cloned animal.
22. The method of Claim 21, wherein purification of the protein of interest is expressed in tissue, cells or bodily secretion of the cloned animal.
23. The method of Claim 22, wherein the tissue, cells or bodily secretion is selected from the group consisting of: milk, blood, urine, hair, mammary gland, muscle, viscera.
24. The method of Claim 23, wherein said viscera is selected from the group consisting of: brain, heart, lung, kidney, pancreas, gall bladder, liver, stomach, eye, colon, small intestine, bladder, uterus and testes.
25. A method of producing a heterologous protein in a transgenic animal comprising the steps of:
- a. combining a genetically engineered genome from an activated donor cell with an activated, enucleated oocyte to thereby form a nuclear transfer embryo, wherein the genome from the activated donor cell encodes the heterologous protein;

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- b. impregnating an animal with the nuclear transfer embryo in conditions suitable for gestation of the nuclear transfer embryo into a cloned animal; and
- c. recovering the heterologous protein from the cloned animal.

5 26. The method of Claim 25, wherein the activated donor cell is in a stage of a mitotic cell cycle selected from the group consisting of: G₁ phase, S phase, and G₂/M phase.

10 27. The method of Claim 25, wherein the activated, enucleated oocyte is in a stage of a meiotic cell cycle selected from the group consisting of: metaphase I, anaphase I, anaphase II and telophase II.

10 Sub B10 28. The method of Claim 25, wherein the genetically engineered genome includes an operatively linked promoter.

15 29. The method of Claim 28, wherein said promoter is selected from the group consisting of: a host endogenous promoter, an exogenous promoter and a tissue-specific promoter.

30. The method of Claim 29, wherein said tissue-specific promoter is selected from the group consisting of: mammary-specific promoter, blood-specific promoter, muscle-specific promoter, neural-specific promoter, skin-specific promoter, hair-specific promoter and urinary-specific promoter.

20 Sub B11 31. A method of enucleating an oocyte having a meiotic spindle apparatus, comprising exposing the oocyte with at least one compound that destabilizes the meiotic spindle apparatus.

32. The method of claim 31, wherein destabilizing the meiotic spindle apparatus further includes destabilizing microtubules, chromosomes, or centrioles.
33. The method of Claim 31, wherein the compound is demecolcine, nocodazole, colchicine, or paclitaxel.
- 5 34. The method of Claim 31, wherein further including altering the temperature, osmolality or composition of medium which surrounds the oocyte.
35. A method of preparing an oocyte for nuclear transfer, wherein the oocyte has a meiotic spindle apparatus, comprising the steps of:
- 10 a. exposing the oocyte to ethanol, ionophore, and/or to electrical stimulation, to thereby obtain an activated oocyte, and
- b. subjecting the activated oocyte to at least one compound that destabilizes the meiotic spindle apparatus, to thereby enucleate the activated oocyte.
36. The method of claim 35, wherein destabilizing the meiotic spindles apparatus includes destabilizing microtubules, chromosomes, or centrioles.
- 15 37. The method of Claim 35, wherein the compound is demecolcine, nocodazole, colchicine, or paclitaxel.
38. The method of claim 35, wherein the activated oocyte is in a stage of a meiotic cell cycle selected from the group consisting of: metaphase I, anaphase I, anaphase II and telophase II.

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